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14. ABSTRACT

Prostate cancer is a heterogeneous disease characterized by diverse outcomes. A subset of patients originally diagnosed with low risk disease go on to be diagnosed with high grade disease. It is important to determine to what extent this is due to undersampling on the initial biopsy, and to what extent low grade disease evolves. In addition, multiple genetic alterations are associated with disease evolution in response to therapy. This project aims to characterize evolution of prostate cancer. Completed work used exome sequencing to evaluate the relationship of low grade foci, high grade foci, and metastases in radical prostatectomy specimens. This work demonstrates that coincident low and high grade disease are distantly related, indicating that an early parental clone can give rise to both low grade and high grade disease. Conversely, lymph node metastases are closely related to high grade cancer. Alterations in the TP53 pathway are associated with high grade disease and lymph node metastases. The observation that alterations identified in lymph node metastases can also be found at low levels in high grade intraprostatic foci led to additional evaluation of high grade index foci, demonstrating considerable heterogeneity across the focus and evidence of commingled subclones. Aims still under investigation are evaluating the relationship between coincident conventional prostate cancer and the aggressive variant ductal adenocarcinoma, and using circulating tumor cells to evaluate the evolution of castrate resistant metastatic cancer from primary foci.

15. SUBJECT TERMS

Cancer genetics, tumor evolution, tumor heterogeneity, prostate cancer, exome sequencing

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Introduction

Prostate cancer is a heterogeneous disease characterized by diverse outcomes. A subset of patients originally diagnosed with low risk disease are later diagnosed with high grade disease. Measures taken to reduce these events depend on to what extent this is due to undersampling on the initial biopsy, and to what extent low grade disease evolves. Whereas it is unclear if low grade, indolent cancer evolves into high grade, aggressive disease, certain genetic alterations are found more often in advanced disease. It is not known if these arise after metastases occur or are found in a subclone of the primary tumor. This project aims to characterize evolution of prostate cancer in both localized and metastatic disease. One aim seeks to evaluate evolution of localized disease to high grade disease or aggressive variants. The second aim seeks to characterize evolution that occurs in the progression of localized prostate cancer to metastatic disease.

Keywords

Cancer genetics, tumor evolution, tumor heterogeneity, prostate cancer, exome sequencing

Overall Project Summary

In March of 2015, in the middle of year 2 of this award, I obtained a new position as an Assistant Clinical Investigator at the National Cancer Institute in Bethesda, Maryland. I left the University of Chicago at the end of June, 2015, and began my new position at NCI in August. The transfer of this award is still on-going as of October 2015.

The research project proposed within this award was divided into two specific aims, with sub aims further delineated within each aim and specific tasks outlined within the statement of work. This portion of the report will be organized by specific aim and according to the tasks outlined in the SOW.

Aim 1: Investigate the molecular relationship between coincident prostate cancer foci.

Aim 1a: *Test the hypothesis that coincident low and high grade foci represent evolution of a single clone.*

In year one we published that coincident low and high grade prostate cancer can be derived from a single clone, but diverge early in their evolution with the majority of mutations private to each focus (VanderWeele et al, Cancer Science, 2014).

While addressing this aim I noted that mutations identified in metastases could also be found at low frequency in the matched high grade focus within the prostate. This led to a collaboration with prostate cancer geneticists at RIKEN and University of Tokyo looking at the genetic heterogeneity within an individual, index focus for men with potentially lethal, localized disease. To complete this work we developed a novel method for banking prostatectomy specimens that preserves the histologic appearance, staining

Aim 2a: *Test the hypothesis that nodal metastases are subclones of high grade prostate cancer foci in the prostate.*

I have completed sequencing and data analysis of tumor DNA from lymph node metastasis from four patients with coincident low and high grade foci in the prostate—two included in *VanderWeele et al, Cancer Science, 2014*, and two in the manuscript currently in preparation addressing intrafocal heterogeneity.

The results of this Aim confirm that nodal metastases are closely related to high grade prostate cancer, and distantly related to low grade prostate cancer. The data are also consistent with the hypothesis that nodal metastases are subclones of high grade prostate foci, for mutations identified in lymph node metastases but not initially identified in high grade foci can be identified at low levels in high grade foci.

Results from this aim are published (*VanderWeele et al, Cancer Science, 2014*). This work fulfills the following subtasks from the SOW: subtask 2.1, subtask 2.3, subtask 3.1.

Aim 2b: *Determine the molecular relationship of CTCs to primary prostate cancer foci.*

To address this aim, I have obtained DNA isolated from CTCs from 7 patients, with biological replicates from three of those patients. In addition, we were able to obtain untreated disease and advanced disease tissue specimens for two of those three patients. My lab has completed DNA isolation, whole genome amplification where needed, and exome sequencing. The analysis is on-going. Because of the overall low quality of the DNA (including whole genome amplification) the analysis is taking longer than expected but still progressing. I am collaborating with the CCR Collaborative Bioinformatics Resource at the NCI to assist with the analysis.

This work addresses subtask 1.2, subtask 1.4, subtask 2.2, subtask 2.4, and subtask 3.2.

As in Aim 1b, at the time the proposal for this project was submitted, next-generation sequencing costs were decreasing rapidly, and thus I proposed doing whole genome sequencing of these samples. Due to persistent high costs, however, instead of deep coverage whole genome sequencing, we are performing deep coverage exome sequencing to identify single nucleotide variants. In addition, since sequencing CTCs requires whole genome amplification, I don't think evaluation of copy number changes is reliable and we will rely instead on single nucleotide variants.

Mentorship

Dr. Walter Stadler is my mentor for clinical and translation research and has continued to promote my professional development. While at the University of Chicago I met with him formally monthly and informally several times throughout the week.

Dr. Yusuke Nakamura, Professor of Medicine, is my basic science mentor. He is one of the pioneers of applying the study of genetic variation to the medical field. While at

University of Chicago I met with him regularly and attend his lab meetings. In addition to mentoring and providing advice, his connections facilitated establishing a collaboration with prostate genomics experts at University of Tokyo and RIKEN to advance the project forward.

Dr. Funmi Olopade is a mentor as an expert in cancer genetics and genetics of hormone-regulated cancers. She is also an important advisor regarding career development.

Key Research Accomplishments for year 2

- Acceptance of five manuscripts, including one on banking of tissue in anticipation of multiregion sequencing, and one comparing ductal and conventional prostate cancer
- Obtaining a new position as Assistant Clinical Investigator at the National Cancer Institute

Changes/problems

In August 2015 I started a position as Assistant Clinical Investigator at the National Cancer Institute. I am in the process of getting the award transferred and recently submitted an updated SOW and budget to reflect this change.

Conclusion

Like other cancers, prostate cancer is a genetic disease that progresses through evolution and accumulation of genetic changes. The genetic heterogeneity in prostate cancer, however, is profound. Multiple distinct foci can be distantly related, possibly even independent. Even within a single focus there is extensive heterogeneity, often with few trunk alterations and many branch and leaf alterations. This implies that many of the mutations found in late stage disease are enrichments of mutations already present in aggressive subclones within the primary lesion. Heterogeneity between histologic ductal cancer and adjacent high grade conventional regions is similar to that between two adjacent conventional regions.

Publications, Abstracts and Presentations

Year 1

1. VanderWeele DJ, Brown CB, Taxy JB, Stadler WM, White KP. Low grade prostate cancer foci diverge early from high grade and metastatic disease. *Cancer Science*. 2014 Aug;105(8):1079-85.
2. Reyes E*, VanderWeele DJ*, Duggan R, Campanile A, Stadler WM, Vander Griend DJ, Szmulewitz R. Quantitative characterization of androgen receptor expression in circulating tumor cells from patients with metastatic castrate resistant prostate cancer. *Journal of Translational Medicine*. 2014 Nov 26;12(1):313.

Year 2

1. Packiam V, Patel SG, Pariser JJ, Richards KA, Weiner AB, Paner GP, VanderWeele DJ, Zagaja GP, Eggener SE. "Contemporary Population-Based Comparison of Localized Ductal Adenocarcinoma and High-Risk Acinar Adenocarcinoma of the Prostate". *Urology*. 2015 Jul 18.
2. VanderWeele DJ, Paner GP, Fleming GF, Szmulewitz RZ. Sustained complete response to cytotoxic therapy and the PARP inhibitor veliparib in metastatic castration resistant prostate cancer – a case report. *Frontiers in Oncology*. 2015 5:169.
3. Gillard M, Tom W, Antic T, Paner GP, Lingen M, VanderWeele DJ. "Next-gen tissue: preservation of molecular and morphological fidelity in prostatectomy specimens". *Am J of Transl Res*. In press.
4. VanderWeele DJ, Stadler WM. "The time for molecular phenotyping of metastatic castrate-resistant prostate cancer is now". *Clinical Advances in Hematology & Oncology*. 2015 13(9), p. 580.
5. McCann S, Jiang Y, Fan X, Wang J, Antic T, VanderWeele DJ, Oto A. "Quantitative Multi-parametric MRI Features and PTEN Expression in Prostate Cancer". *Am J of Roentgenology*. In press.

Inventions, Patents and Licenses

Nothing to report

Reportable Outcomes

Nothing to report

Other Achievements

The data from this work, and the protected time afforded by this award, has led to additional projects looking at correlations with MRI features and specific genomic alterations, including subclonal loss of PTEN. This has led to acceptance of an initial manuscript. I am also a co-PI for a primary aim of a PO1 application which is following up on these initial studies.

I have accepted a position as an Assistant Clinical Investigator at the National Cancer Institute. I have selected a postbaccalaureate student and am actively recruiting for a postdoctoral position.

Opportunities for Training

As per the SOW, I have attended the following courses:

From year 1:

Responsible Conduct of Research

Ecology and Evolution: Principles of Population Genetics I

Ecology and Evolution: Genomic Evolution

From year 2:

Human Genetics: Introduction to Probability and Statistics for Geneticists

From year 3:

Statistics: Statistical Methods and their Applications

The Ecology and Evolution: Fundamentals of Molecular Evolution course from year 1, and Intro to Statistical Genetics and Population Genetics II from year 2 were no longer offered.

Opportunities for Professional Development

I had the opportunity to attend and participate in international conferences during the second year of this award. I presented a poster on my work trying to optimize banking of prostate tissue to conduct higher quality studies on prostate cancer genomics at the Prostate Cancer Foundation Scientific Retreat. This meeting brings together the brightest minds in prostate cancer research along with representatives from industry and policy.

I presented a poster on work looking at the loss of PTEN, a genomic event that is enriched in advanced prostate cancers, at the GU cancer symposium. This is a more clinically oriented meeting affiliated with the American Society of Clinical Oncology. I also attended the ASCO annual meeting.

I was the institutional PI for several clinical trials, including a trial evaluating neoadjuvant chemotherapy for high risk prostate cancer, which is run through the CALGB/Alliance clinical trials consortium, and a trial using an AURKA inhibitor for patients with neuroendocrine or small cell cancer of the prostate. Patients could have been diagnosed with these aggressive variants initially or have had conventional cancer that evolved into these more aggressive phenotypes. As my role as institutional PI I also participated in the fall CALGB/Alliance meetings.

References

VanderWeele DJ, Brown CB, Taxy JB, Stadler WM, White KP. Low grade prostate cancer foci diverge early from high grade and metastatic disease. Cancer Science. 2014 Aug;105(8):1079-85.

Appendices

None.